stress conditions [1]. The transporter contributes to virulence of pathogens such as *Staphylococcus aureus* and *Helicobacter pylori*. We utilize PutP of *Escherichia coli* as a model to explore structure and molecular mechanism of function of SSSF proteins.

Here, we present a model of the helix bundle of PutP obtained by molecular modeling constrained by experimentally determined intramolecular distances and template restraints derived from the ten-helix core of the vSGLT crystal structure [2]. For this purpose, DEER distance measurements between spin labels attached to helix ends were conducted and mean interspin distances were determined. Fitting algorithm based on matrix geometry in combination with prediction of spin label conformations by a rotamer library approach [3] resulted in an ensemble of helix bundle structures. The central structure of the ensemble showed a core structure with a fold similar to that of the vSGLT template. Furthermore, analysis of spin label motility and environmental polarity by cwEPR yielded information on secondary structure elements and structural rearrangements of external loop (eL) 9 of PutP upon sodium and/or l-proline binding. The results support the idea that eL9 controls access to the sodium and/or l-proline binding site(s) similar as previously proposed for eL 4 of LeuT [4].

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4P6

Mitochondrial carrier structure and diseases

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To date eleven disorders are known to be caused by defects of mitochondrial carriers, a family of proteins that shuttle a variety of metabolites across the inner mitochondrial membrane. Mutations of mitochondrial carrier genes are responsible for carnitine/acylcarnitine carrier deficiency, ornithine carrier deficiency (HHH syndrome), aspartate/glutamate isoform 1 deficiency (global cerebral hypomyelination), aspartate/glutamate isoform 2 deficiency (CTLN2 and NICCD), Amish microcephaly, early epileptic encephalopathy, congenital sideroblastic anemia, PiC deficiency, ADP/ATP carrier isoform 1 deficiency, neuropathy with bilateral striatal necrosis and adPEO (autosomal dominant progressive external ophthalmoplegia). Structural, functional and bioinformatics studies have revealed the existence in mitochondrial carriers of a substrate-binding site in the internal carrier cavity, of two gates that close the cavity alternatively on the matrix or cytosolic side of the membrane, and of two sets of prolines and glycines in the six transmembrane a-helices located strategically between the substrate-binding site and the two gates. The key role played by these mitochondrial carrier areas is supported by the observation that they host most of the disease-causing missense mutations of the mitochondrial carriers.

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4P7

Insights into the mechanism of the Na^+/Ca^{2+} exchanger from atomistic molecular dynamics simulations

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Na^+/Ca^{2+} exchangers (NCX) and potassium-dependent Na^+/Ca^{2+} exchangers (NCKX) are two related families of transporters involved in Ca^{2+} signaling that function by extruding cytosolic Ca^{2+} (and K^+ for the potassium-dependent transporter) in exchange for extracellular Na^+ [1]. Previous studies have established that this exchange process is electrogenic and with a defined stoichiometry, and have identified specific acidic aminoacids believed to be crucial for ion binding and translocation [1–3]. Recently the crystal structure of the NCX from *Methanococcus jannaschii* was determined at 1.9 Å resolution [4], revealing an intriguing transmembrane topology consisting of inverted structural repeats, and the presence of four putative ion binding sites formed by highly conserved residues. Notwithstanding these groundbreaking insights, based on the structure alone several ion occupancy states can be hypothesized that would be compatible with the experimental exchange stoichiometry. Moreover, in the crystal the protein adopts a unique outward facing conformation, which does not immediately explain how ion binding to the protein facilitates the necessary outward-to-inward conformational transition. Here, we use extensive molecular simulations and molecular modeling to investigate the occupancy and specificity of the ion binding sites in NCX_Mj, and the microscopic mechanism by which Na^+ and Ca^{2+} are exchanged across the membrane.

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4P8

The evolutionary history of membrane-integral pyrophosphatases supports Na^+ as the ancestral coupling ion in membrane bioenergetics

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